Tracking trails by cracking codes

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Chapter 10

Summary of Conclusions

The main objective of this thesis was to examine the evolutionary diversification and biogeography of some polar and cold-temperate seaweeds. Distribution patterns and dispersal routes were investigated in three species exhibiting a bipolar disjunction and one with an amphi-Atlantic distribution, by means of a suite of molecular data appropriate to the geographic scale of the question. In addition, the evolutionary diversification of an essentially cold-water group (Ulotrichales, Chlorophyta) and phylogenetic relationships among taxa within the group were examined. The main conclusions from this thesis can be summarized as follows.

Bipolar Disjunctions

The bipolar disjunctions in the seaweed species *Acrosiphonia arcta*, *Urospora penicilliformis* and *Desmarestia viridis* are of recent origin, possibly as recent as the last glacial maximum (ca 18,000 years ago). This is evidenced by a comparison of the fast evolving rDNA internal transcribed spacer (ITS) sequences, which show little or no divergence among Arctic and Antarctic isolates of each of the three species (Chapters 2 and 4). Trans-equatorial dispersal during the mid-Miocene cooling period, when the tropical belt was somewhat contracted and cooler for the first time since the Cretaceous, is not supported. The most likely dispersal routes of *A. arcta* were partially unraveled by means of a restriction map analysis of the even faster evolving rDNA intergenic spacer (IGS) and Random Amplified Polymorphic DNAs (RAPDs) (Chapter 3). *Acrosiphonia arcta* crossed the equator in the Pacific Ocean, but the dispersal directionality across the equator (north to south or south to north) could not be inferred from the data. After the last ice cap had disappeared from the Arctic Ocean, *A. arcta* colonized the North Atlantic via the Bering Strait. This is shown by the close relationship between the North Pacific and West Atlantic isolates and the increased distance between the North Pacific and East Atlantic isolates. It is further concluded that Disko Island (Greenland) was colonized by an independent dispersal event. Present-day distribution patterns of *A. arcta* in the North Atlantic are of relatively recent origin, i.e., within the time frame of thousands rather than millions of years, and dispersal events have played an important and measurable role.

Amphi-Atlantic Distribution in *Phycodrys rubens*

ITS sequence comparisons in *Phycodrys rubens* indicate that eastward trans-Arctic dispersal into the North Atlantic has taken place twice (Chapter

6), as was the case for *A. arcta*. The first invasion occurred shortly after the submergence of the Bering landbridge (3-3.5 Ma ago). Both western and eastern North Atlantic coasts were colonized at that time. Later, during the Pleistocene glaciations, *P. rubens* probably became locally extinct along the West Atlantic coasts, but survived on the eastern side of the ocean. After the last ice age, the western Atlantic coasts were colonized by Pacific *Phycodrys* plants a second time. Both ITS and allozyme data indicate the presence of distinct genotypes on both sides of the Atlantic basin. The fact that the American Atlantic coasts were only recently invaded by Pacific *Phycodrys* plants may explain why these plants are not as well adapted to the prevailing temperature conditions as their European counterparts. ITS sequences, Rubisco spacer sequences and allozymes further show that two genetically distinct groups are found along the European coasts, one in the North Sea and Baltic and one along the more open oceanic coasts. It is hypothesized that the isolation of some East Atlantic populations in ice-free refugia during the Pleistocene glaciations has resulted in these distinct genotypes. The absence of hybrids of the two types in areas where they co-occur (the Skagerrak and Kattegat area) suggest that the two genetic types are not interfertile and represent cryptic species (Chapter 7). Finally, the lack of correlation between genetic groupings and known salinity ecotypes occurring in the North Sea and Baltic "neck", indirectly supports the extreme recency of events in the Baltic area.

Long-distance Dispersal in Benthic Macrophytes

Past generalizations about the poor dispersability of benthic macrophytes were based primarily on comparisons with the pelagic larval stages of various marine animals. By that comparison seaweeds are indeed poor dispersers. Molecular data now indicate that seaweeds do successfully disperse over large distances -- but they probably do so via drifting thalli, rafting on various debris or as epi- or endophytic microthalli growing in larger rafting algae. In the bipolar disjunct species investigated in this thesis, the microthallus stage is undoubtedly of great importance for long-range dispersal.

Relatively Recent Divergence of Some Cold-Water Taxa

Relatively low levels of ITS sequence difference among a group of essentially cold-water genera belonging to the Ulotrichales (i.e. *Acrosiphonia*, *Spongomorpha*, *Urospora* and *Ulothrix*), suggest that the first divergence among these genera was initiated during the mid-Miocene cooling period (10-15 Ma ago) (Chapter 4). Analysis of ITS sequences among three *Acrosiphonia* species, *A. arcta*, *A. sonderi* and *A. coalita*, further indicates that these species are part of just a single species (*A. arcta*) or constitute a species-complex.

Group I Introns

Group I introns were discovered at the 3'-end of the 18S gene of *U. penicilliformis* (Chapter 5) and at the approximate position 1210 of the 18S gene in *U. penicilliformis* and *Ulothrix implexa* (van Oppen, unpubl.). Although it has been shown that group I introns are sometimes inherited through common ancestry, lateral transfer of group I introns also occurs. Evidence for this comes from the observation that closely related introns are distributed across different genomes and unrelated organisms and that the "mid-position" (ca position 1210 in the rDNA 18S gene) *U. penicilliformis* and *U. implexa* introns show hardly any sequence similarity with other green algal "mid-position" introns (van Oppen, unpubl.) Not all *U. penicilliformis* strains have this "mid-position" intron, suggesting that they have either lost it or that it has been acquired by lateral transfer.

The detection of group I introns in algae is important in terms of understanding some of the processes underlying their genome evolution. Some group I introns are mobile. As long as these group I introns stay functionally silent, they can multiply and spread through the genome by "intron homing" and behave as "selfish DNA", thus influencing molecular evolution. If splicing is hindered, the intron will alter the reading frame of protein-coding genes and the end-product of ribosomal genes, and may have drastic phenotypic effects. Similarly, imprecise "reverse splicing" of group I introns may result in the deletion or addition of bases.

Applicability of the Different Methods

The articles in this thesis span a broad range of time and space. This required the use of a range of molecular data able to provide the appropriate levels of resolution over a sliding scale from phylogeny to population genetics. Both conservative genes (rDNA 18S) and fast evolving spacers (rDNA ITS, rDNA IGS, Rubisco) were used, as well as allozymes and RAPD "fingerprints".

Beginning at the conservative end of the scale, the variable region V4 of the rDNA 18S gene, which evolves more slowly than the rDNA ITS regions, worked well for resolving relationships among orders in the Ulvophyceae (Chlorophyta) (Chapter 4). ITS sequences worked well among closely related seaweed species (Chapter 4) and for intraspecific comparisons in some seaweeds (Chapter 6). The degree to which intraspecific comparisons were possible depended upon the time and geographic scale of the separation experienced by the biogeographic populations. If elapsed time was short (roughly, < 1 Ma) the ITS did not provide sufficient resolution (Chapters 2 and 4). The Rubisco spacer behaved similarly to ITS in terms of resolving power at this level. The rDNA IGS is definitely a population-level marker. Investigation of IGS types in *A. arcta* populations revealed discrete groups with no evidence for intra-individual variation, but no population structure could be detected within the North Atlantic (Chapter 3). Analysis of RAPDs (a more qualitative population-level marker) was found to work well for broadscale intraspecific biogeographic comparisons, but

collapsed at more local scales (Chapter 8). A discussion about the uses and limitations of RAPDs from a technical as well as theoretical point of view is presented in detail in Chapter 8. It is concluded that the dominant Mendelian nature of RAPDs and the effects of primer binding site competition lead to a number of limitations in the data that weaken its resolving power when attempts are made to apply the data in a population genetic study. Allozyme variation was assessed in *P. rubens* (Chapter 7). Allozymes were fixed for a single allele at all investigated loci. Among the over 200 *P. rubens* plants screened not a single individual was heterozygous. It is hypothesized that the lack of heterozygotes reflects a bottleneck causing one allele to become fixed at each locus by random genetic drift, or that reduced within-population variation is the result of asexual reproduction and inbreeding. Only by the application of single-locus DNA fingerprinting techniques it is likely that this question can be resolved.

Marine Biodiversity

Studies of the type presented in this thesis play a significant role in understanding geological and paleoclimatic, as well as present-day influences that structure biogeographic patterns. Molecular studies can be used to track dispersal and distinguish the relative role of vicariance (i.e. identification of the correct time frame), and provide estimates of the genetic diversity on several levels, e.g. discovery of cryptic species and high genetic diversity within single species and species-complexes. This type of information is of great importance for marine biodiversity issues (Chapter 9).